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ORIGINAL ARTICLE

Effect of milk fat content on the performance of ohmic heating for inactivation of *Escherichia coli* O157:H7, *Salmonella enterica* Serovar Typhimurium and *Listeria monocytogenes*

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Keywords

conventional heating, electrical conductivity, milk fat, ohmic heating, pathogen inactivation, protective effect.

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Abstract

Aims: The effect of milk fat content on ohmic heating compared to conventional heating for inactivation of food-borne pathogens was investigated.

Methods and Results: Sterile cream was mixed with sterile buffered peptone water and adjusted to 0, 3, 7, 10% (w/v) milk fat content. These samples with varying fat content were subjected to ohmic and conventional heating. The effect of milk fat on temperature increase and electrical conductivity were investigated. Also, the protective effect of milk fat on the inactivation of foodborne pathogens was studied. For conventional heating, temperatures of samples increased with time and were not significantly ($P > 0{\cdot}05$) different regardless of fat content. Although the inactivation rate of *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *L. monocytogens* decreased in samples of 10% fat content, a protective effect was not observed for conventional heating. In contrast with conventional heating, ohmic heating was significantly affected by milk fat content. Temperature increased more rapidly with lower fat content for ohmic heating due to higher electrical conductivity. Nonuniform heat generation of nonhomogeneous fat-containing samples was verified using a thermal infrared camera. Also, the protective effect of milk fat on *E. coli* O157:H7 and *Listeria monocytogenes* was observed in samples subjected to ohmic heating.

Conclusions: These results indicate that food-borne pathogens can survive in nonhomogeneous fat-containing foods subjected to ohmic heating. Therefore, more attention is needed regarding ohmic heating than conventional heating for pasteurizing fat-containing foods.

Significance and Impact of the Study: The importance of adequate pasteurization for high milk fat containing foods was identified.

Introduction

Ohmic heating is a novel heating technology using electric current to generate heat inside of food. It has immense potential for achieving rapid and uniform heating in foods, providing microbiologically safe and high quality foods. Because of these advantages, ohmic heating can be used in various food industries. Possible applications for ohmic heating in the food industry include blanching, evaporation, dehydration, fermentation, extraction, sterilization and pasteurization (Ramaswamy *et al.* 2014). Ohmic heating can be used for solid-liquid

food mixtures because liquid and solid phases can be heated simultaneously (Lee *et al.* 2013). Regarding pasteurization, the thermal and nonthermal effect can be shown for microbial inactivation with ohmic heating (Park and Kang 2013). However, the nonthermal effect of ohmic heating is still an area of controversy. Some research indicates that ohmic heating may confer mild nonthermal cellular damage due to the presence of the electric field (Knirsch *et al.* 2010). On the other hand, other research indicates that ohmic heating showed no significant difference compared to conventional heating (Palaniappan *et al.* 1992). Nevertheless, it is certain that the principal mechanism of microbial inactivation in ohmic heating is the thermal effect.

Electrical conductivity (σ) is the main critical factor determining the rate of ohmic heating. There are critical conductivity values below 0·01 Siemens/m (S/m) and above 10 S/m where ohmic heating is not applicable (Piette *et al.* 2004). Although in some foods electrical conductivity is not temperature-dependent, for the most part electrical conductivity increases with increasing temperature (Palaniappan and Sastry 1991). Electrical conductivity is affected by the nature of ions, ionic movement and viscosity of the liquid. Electrical conductivity is also affected by moisture content, starch gelatinization, applied frequency, voltage, type of food, and type and cut of meat (Pongviratchai and Park 2007; Sarang *et al.* 2008).

Fat is one of the three major nutrients and performs many roles in the human body. Fat content in food varies considerably according to differences even within a type of food. Milk fat is the primary component of cream and milk is divided into three types according to fat content: whole milk, low-fat milk, and skimmed milk (Adebamowo *et al.* 2008). The effect of fat content in various foods on inactivation of pathogens has been of interest. Some research-investigations have reported that fat content had no significant effect on the inactivation of pathogens. Byelashov *et al.* (2010) identified no differences in the *Escherichia coli* O157:H7 inactivation rate between ground beef knuckle meat (approx. 5% fat) and ground beef shoulder meat (approx. 15% fat) subjected to a circulating water bath (75°C). Kotrola and Conner (1997) and Stoltenberg *et al.* (2006) also reported that fat content had no significant effect on the inactivation of *E. coli* O157:H7 when processed by thermal treatment. Inactivation of *E. coli* O157:H7, salmonellae, *Campylobacter jejuni*, *Listeria monocytogenes* and *Staphylococcus* spp. in raw ground beef was not influenced by the fat content when treated with gamma irradiation (Clavero *et al.* 1994; Monk *et al.* 1994). Inactivation of *L. monocytogenes* Scott A was not significantly different among whole, 2%, and skimmed milk when treated with pulsed electric field (Reina *et al.* 1998).

In contrast with these previous research-investigations, the reduction of pathogens was affected by fat content in other studies. Heat resistance of *Salm*. Typhimurium DT 104 in beef increased with higher fat levels (Juneja and Eblen 2000). The inactivation rate of *Listeria innocua* decreased with increasing fat content when treated by thermo-sonication (Bermúdez-Aguirre and Barbosa-Cánovas 2008). Juneja and Eblen (2000) and Bermúdez-Aguirre and Barbosa-Cánovas (2008) used a shaking water bath or oil bath for conventional heating treatment. However, no previous study has reported the effect of fat content on ohmic heating compared to conventional heating.

Escherichia coli O157:H7, *Salm*. Typhimurium, and *L. monocytogenes* are pathogenic micro-organisms commonly reported in food borne out-breaks traced to dairy farms. Among farm environmental samples, 3·76, 6·51 and 0·72% were positive for *Salmonella* spp., *L. monocytogenes* and *E. coli* O157:H7, respectively (Murinda *et al.* 2004). The effect of milk fat content on temperature increase and electrical conductivity was observed for both ohmic and conventional heating methods. Moreover, the protective effect of milk fat on *E. coli* O157:H7, *Salm*. Typhimurium and *L. monocytogenes* was investigated when bacterial cultures were inoculated after reaching the target temperature. The objective of the present study was to investigate the comparative effect of milk fat content on the efficacy of ohmic and conventional heating.

Materials and methods

Bacterial cultures and cell suspension

Three strains each of *Escherichia coli* O157:H7 (ATCC 35150, ATCC 43889 and ATCC 43890), *Salmonella* Typhimurium (ATCC 19585, ATCC 43971 and DT 104) and *Listeria monocytogenes* (ATCC 19111, ATCC 19115 and ATCC 15313) were obtained from the bacterial culture collection of the School of Food Science, Seoul National University (Seoul, South Korea). Cultures were produced as follows: a single colony cultivated from frozen stocks on tryptic soy agar (TSA; Difco, Becton, Dickinson, Sparks, MD) was inoculated into 5 ml of tryptic soy broth (TSB; Difco, Becton, Dickinson), incubated at 37°C for 24 h, collected by centrifugation at 4000 ***g*** for 20 min at 4°C and washed three times with 0·2% peptone water (PW; Bacto, Becton, Dickinson). The final pellets were resuspended in 0·2% PW, corresponding to approx. 10^8–10^9 CFU ml^{-1}. Afterwards, suspended pellets

of the three pathogens were combined to comprise a mixed culture cocktail containing approximately equal numbers of cells of each strain of *E. coli* O157:H7 (10^7 CFU ml^{-1}), *Salm*. Typhimurium (10^7 CFU ml^{-1}) and *L. monocytogenes* (10^6 CFU ml^{-1}). The target temperature for the inactivation of *E. coli* O157:H7, *Salm*. Typhimurium and *L. monocytogenes* was 60°C.

Sample preparation and inoculation

Sterile buffered peptone water (BPW; Difco) was used in this experiment**.** Sterile cream containing 37% fat and emulsifier were purchased from a local grocery store (Seoul, South Korea). BPW was mixed with pasteurized cream to achieve fat contents of 0% (w/o cream) 3%, 7%, and 10% (w/v). Samples were mixed using a magnetic stirrer and stir bar. A mixed-culture cocktail (0·2 ml) was inoculated into 25 ml of prepared sample tempered to room temperature before treatment in experiments involving temperature increase. On the other hand, mixed-culture cocktail was inoculated into the sample after it reached target temperature for experiments performed at a fixed temperature.

Conventional heating treatment

For conventional heating, a constant-temperature water bath (BW-10G; Jeio Tech, Seoul, South Korea) was used in this study. The conventional heating chamber was made of stainless steel (2 × 15 × 6 cm) of 0·2 cm thickness. Twenty-five millilitre of sample was placed into the conventional heating chamber and inoculated with 0·2 ml of mixed culture cocktail. Temperature of the water bath was fixed at 75°C. A fibre optic temperature sensor (FOT-L; FISO Technologies Inc., Québec, QC, Canada) connected to a signal conditioner (TMI-4; FISO Technologies Inc.) was used to measure the temperature in the middle of the sample. Prepared sample was treated for 0, 30, 60, 90, 120, 150 and 180 s.

To identify the protective effect of fat content on pathogen inactivation, experiments at a fixed temperature (62°C) were performed in a water bath. After reaching target temperature (60°C), 0·2 ml of mixed-culture cocktail was inoculated into the sample and treated for 2 min. Treatment temperature (60°C) and time (2 min) were chosen based on a preliminary experiment to obtain moderate inactivation of pathogens.

Ohmic heating system

The ohmic heating system (Fig. 1) consisted of a function generator (catalogue number 33210A; Agilent Technologies, Palo Alto, CA), a precision power amplifier

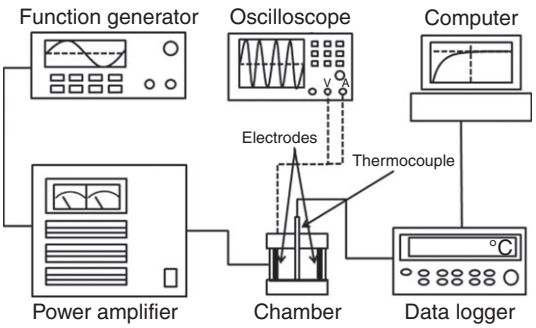

Figure 1 Schematic diagram of the ohmic heating system at Seoul National University (Seoul, Korea).

(catalogue number 4510; NF Corp., Yokohama, Japan), a two-channel digital-storage oscilloscope (catalogue number TDS2001C; Tektronix, Inc., Beaverton, CO), a data logger (catalogue number 34970A; Agilent Technologies) and an ohmic heating chamber. The function generator produced various waveforms at frequencies from 1 to 10 MHz and a maximum output level of 5 V. The signals generated through the power amplifier were amplified up to a maximum output of 141 V alternating current (AC). The signals expanded by the power amplifier were delivered to each of two titanium electrodes. The two-channel digital storage oscilloscope was used to measure signals, including waveform, frequency, voltage and current. Temperature was controlled by LABVIEW software (National Instrument, Austin, TX). K-type thermocouples were inserted at the centre of the ohmic heating chamber and temperatures were recorded at 0·6-s intervals by a data logger and function generator operated according to whether the target temperature was reached or not. The distance between the two electrodes was 2 cm, and the cross-sectional area was 60 cm^2.

Ohmic heating treatment

For ohmic heating experiments, an ohmic heating system with a 20 kHz frequency and sine waveform were used. The ohmic heating chamber was made of polyvinyl chloride (2 × 15 × 6 cm) of 0·5 cm thickness. Twenty-five millilitre of sample was placed into the ohmic heating chamber and inoculated with 0·2 ml of mixed-culture cocktail before treatment. Each sample was treated for 0, 10, 20, 30, 35, 40, 45 and 50 s with 19·2 V cm^{-1} ohmic heating. To identify the protective effect of fat content on pathogen inactivation, each sample was placed into the ohmic heating chamber and treated at a fixed temperature (60°C). Temperature was controlled by LABVIEW software (National Instrument). The electric field was fixed at 9·6 V cm^{-1} after reaching the target temperature to maintain temperature stability. The function generator

operated according to whether the target temperature was reached or not (on-off system). After reaching targeted temperature (60°C), 0·2 ml of mixed-culture cocktail was inoculated into the sample and treated for 2 min.

Electrical conductivity measurement

Electrical conductivity of sample was determined from current and voltage data (Palaniappan and Sastry 1991) and calculated as follows (equation 1):

$$\sigma = \frac{\mathrm{LI}}{\mathrm{AV}} \quad (1)$$

where σ is the electrical conductivity, L is the distance between electrodes (m), I is the intensity of the current, A is the cross-sectional area of the electrodes and V is the voltage. Voltage and current were measured using the two channel digital storage oscilloscope.

Temperature distribution measurement

Surface temperature distribution of samples with 0% and 10% fat content was measured by means of a thermal infrared camera (FLIR, E60). The camera has a resolution of 320 × 240 pixels with a thermal sensitivity of 0·05°C. Sterile cream was mixed with sterile BPW to achieve 10% fat content to observe temperature distribution of fat-containing samples. Each sample was treated with 19·2 V cm^{-1} ohmic heating until reaching 40, 50, 60 and 70°C. The surface of samples was captured.

Microbial enumeration

For microbial enumeration, each treated 25 ml sample was immediately transferred into a sterile stomacher bag (Labplas, Inc., Sainte-Julie, QC, Canada) containing 225 ml of 0·2% PW and homogenized for 2 min in a stomacher (Easy Mix; AES Chemunex, Rennes, France). After homogenization, 1 ml aliquots withdrawn from stomacher bags were serially diluted 10-fold in 0·2% PW and 0·1 ml of appropriate diluents were spread-plated onto each selective medium. Sorbitol MacConkey (SMAC) agar (Difco), xylose lysine deoxycholate (XLD) agar (Difco) and Oxford agar base (OAB; Difco) with selective supplement (Bacto Oxford antimicrobial supplement; Difco) were used as selective media for enumeration of *E. coli* O157:H7, *Salm*. Typhimurium and *L. monocytogenes*, respectively. Where low levels of surviving cells were expected, 1 ml aliquots withdrawn from stomacher bags were divided between four plates of each medium and spread plated. After all plates were incubated at 37°C for 24–48 h, colonies were counted.

Statistical analysis

All experiments were duplicate-plated and replicated three times. All data were analysed by the analysis of variance (ANOVA) procedure of the Statistical Analysis System (SAS Institute, Cary, NC) and mean values were separated using Duncan's multiple-range test. Significant differences in the processing treatments were determined at a significance level of $P = 0{\cdot}05$.

Results

The effect of fat content on the temperature increase and electrical conductivity

The temperature histories of samples subjected to ohmic heating with fat content ranging from 0 to 10% are shown in Fig. 2a. Temperature increased with increasing treatment time at each fat content sample. The heating rate of samples with 3% fat content did not differ from those of 0% fat content. However, the heating rate of samples with 7% fat content decreased significantly ($P < 0{\cdot}05$) followed by 10% fat content. Temperature curves including error bars for samples of 3, 7 and 10% fat content did not overlap with each other. The electrical conductivity of samples with fat content ranging from 0 to 10% subjected to ohmic heating is shown in Fig. 3. Electrical conductivity increased with increasing time. For each time interval, electrical conductivity was smaller as fat content increased. In contrast with ohmic heating, there was no significant effect ($P > 0{\cdot}05$) of fat content (0–10%) on the temperature increase of conventional heated samples. Temperature curves, including error bars, for those of varying fat content (0–10%) are shown in Fig. 2b; these curves overlap each other. In contrast with ohmic heating, the rate of conventional heating decreased as temperature approached the target temperature. The different pattern and heating rate of conventional compared to ohmic heating resulted from the existence of external heat source in the case of conventional heating. Significant differences ($P = 0{\cdot}05$) between temperature curves both in ohmic and conventional heating were verified using statistical analysis.

Temperature distribution of ohmic heated nonhomogeneous fat-containing samples

Thermal images of untreated and ohmic treated sample upper surfaces are shown in Fig. 4. In the thermal images, the red and blue sections represent the highest and lowest temperatures, respectively. The yellow section in the thermal images represents medium temperatures. The blue section in (A1) and (B1) represents samples in

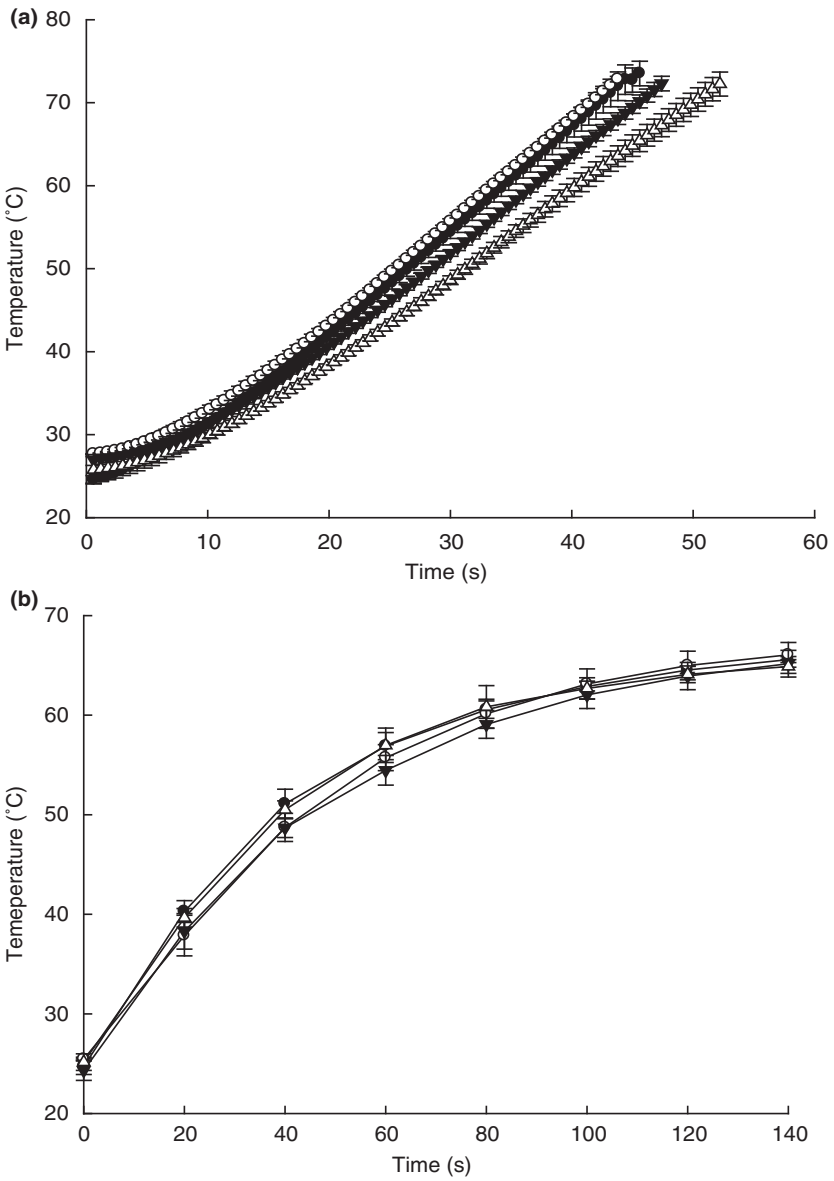

Figure 2 Temperature histories of samples subjected to (a) ohmic and (b) conventional heating with 0% (●), 3% (○), 7% (▼) and 10% (△) fat content. The results are means from three experiments, and error bars indicate standard errors.

the rectangular ohmic heating chamber. The red section in (A2) ~ (A5) and (B2) ~ (B5) represents samples subjected to ohmic heating. The thermal images of each 40°C sample were enlarged to depict differences clearly (A6 and B6). Thermal images of ohmic treated fat-containing samples revealed differences in temperature distribution.

The effect of the fat content on the inactivation of *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* corresponding to temperature increase of ohmic heating

The survival of *E. coli* O157:H7, *Salm*. Typhimurium and *L. monocytogenes* in the samples with varying fat content corresponding to temperature histories (Fig. 2a) is shown in Fig. 5. Regardless of the type of pathogen, ohmic heating in samples of lower, rather than higher, fat content achieved greater reduction for the same treatment time. Also, ohmic heating of lower fat content samples required less time for pathogens to decrease below the detection limit than for those of a higher fat content. For *E. coli* O157:H7, 40 and 50 s were needed to decrease the population below the detection limit for 0% and 10% fat content samples, respectively. For *Salm*. Typhimurium, 35 and 45 s were needed to decrease populations below the detection limit for 0% and 10% fat content samples, respectively. Finally for *L. monocytogenes*, 40 and 50 s were needed to decrease populations below the detection limit for 0% and 10% fat content samples, respectively.

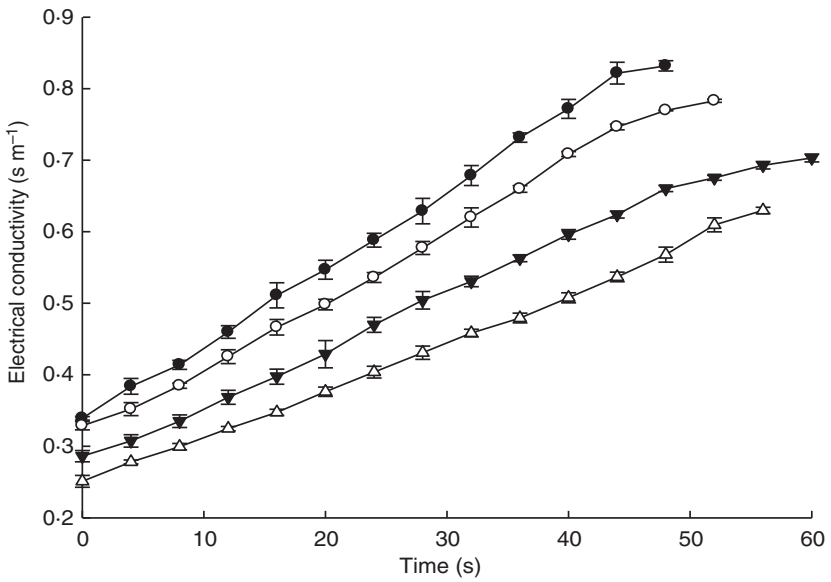

Figure 3 Electrical conductivity histories of samples subjected to ohmic heating with 0% (●), 3% (○), 7% (▼), and 10% (△) fat content. The results are means from three experiments, and error bars indicate standard errors.

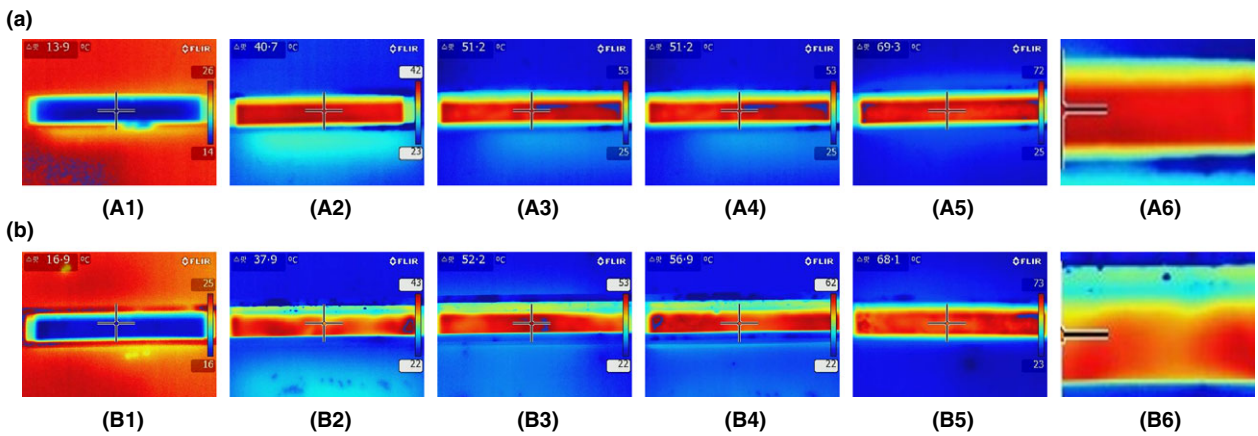

Figure 4 Surface temperature distribution of samples subjected to ohmic heating with (a) 0% fat content and (b) 10% non-homogeneous fat content. (a). Thermal images of samples with 0% fat content; (A1) untreated, temperatures of (A2) 40°C, (A3) 50°C, (A4) 60°C, (A5) 70°C and (A6) enlarged A2. (b) Thermal images samples with 10% fat content; (B1) untreated, temperatures of (B2) 40°C, (B3) 50°C, (B4) 60°C, (B5) 70°C and (B6) enlarged B2.

The effect of the fat content on the inactivation of *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* corresponding to temperature increase of conventional heating

The survival of *E. coli* O157:H7, *Salm*. Typhimurium and *L. monocytogenes* in the samples with varying fat content corresponding to temperature histories (Fig. 2b) is shown in Fig. 6. The reduction of *E. coli* O157: H7, *Salm*. Typhimurium and *L. monocytogenes* was not different among the samples with 0, 3 and 7% fat content. Although reduction of three pathogens decreased in the samples with 10% fat content, survival curves including error bars were overlapped with survival curves in the samples with 0, 3 and 7% fat content. The survivals of *E. coli* O157:H7, *Salm*. Typhimurium and *L. monocytogenes* in the samples with varying fat content (0 ~ 10%) were not significantly different ($P > 0{\cdot}05$).

The protective effect of the fat content on the inactivation of *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes*

The reduction of *E. coli* O157:H7, *Salm*. Typhimurium and *L. monocytogenes* in the samples with 0% fat content were moderate (2–4 log reduction CFU ml^{-1}) in the preliminary experiments (60°C, 2 min). The reduction of pathogens in samples subjected to ohmic heating for a

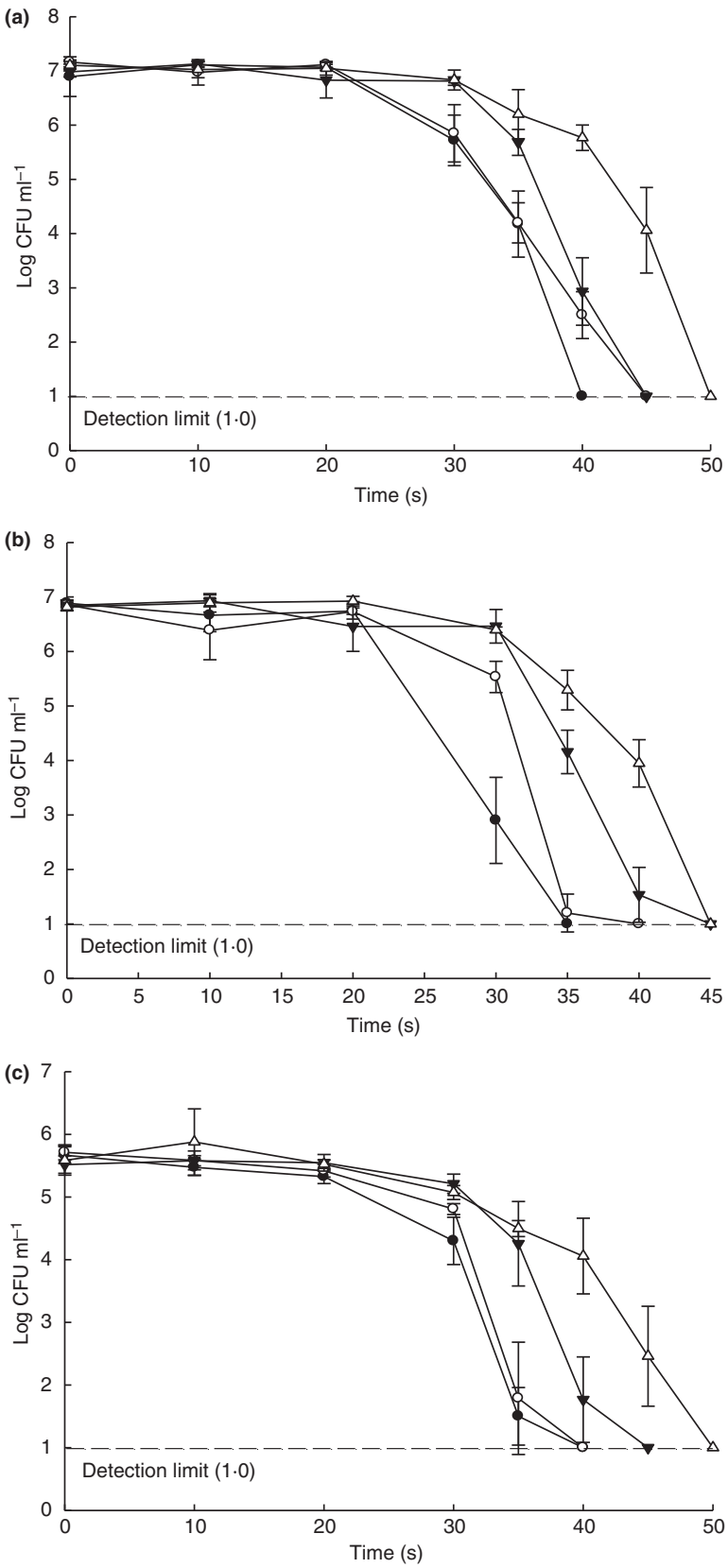

Figure 5 Survival curves of (a) *Escherichia coli* O157:H7, (b) *Salmonella* Typhimurium and (c) *Listeria monocytogenes* corresponding to temperature increase (Fig. 2a) in samples subjected to ohmic heating with 0% (●), 3% (○), 7% (▼) and 10% (△) fat content. The results are means from three experiments, and error bars indicate standard errors.

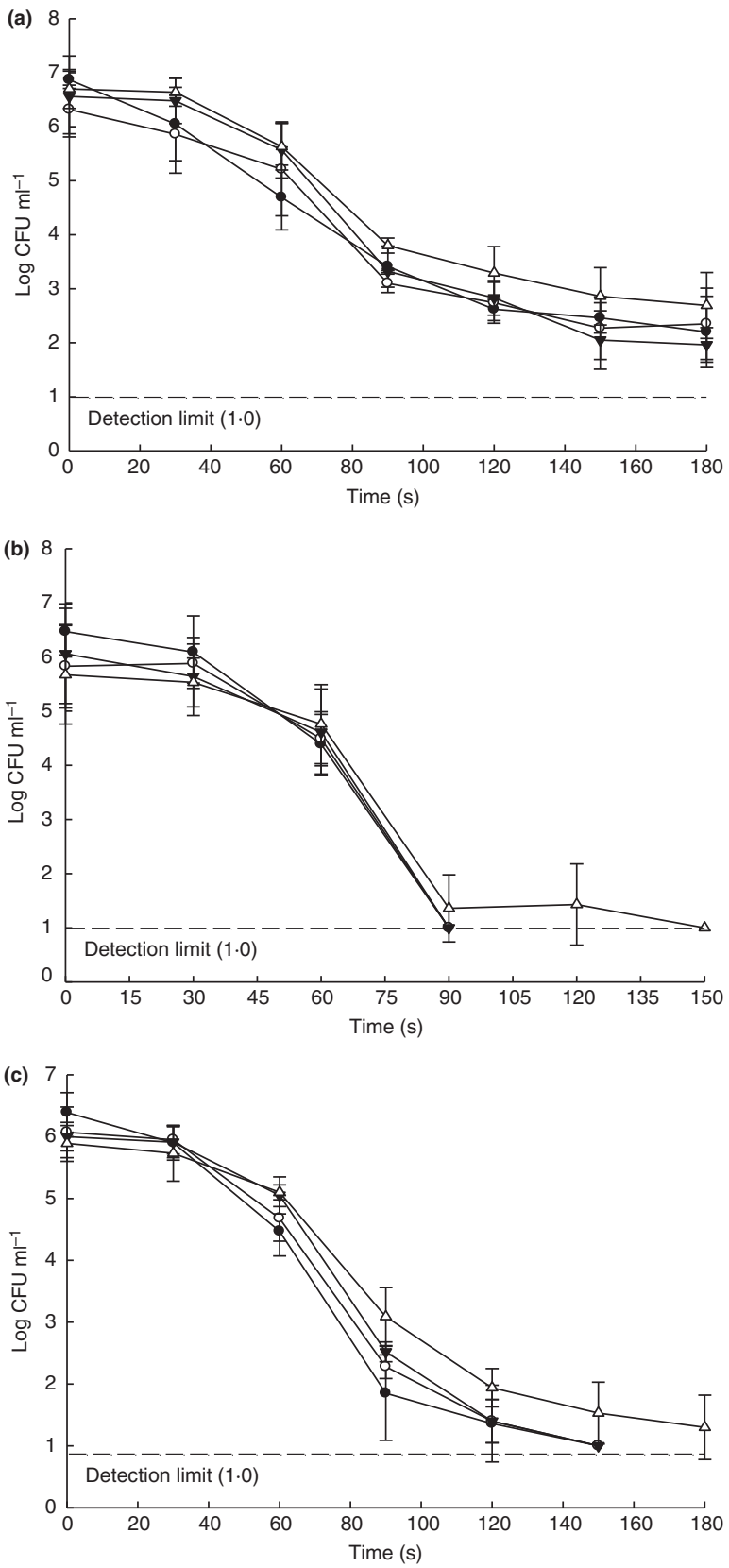

Figure 6 Survival curves of (a) *Escherichia coli* O157:H7, (b) *Salmonella* Typhimurium and (c) *Listeria monocytogenes* corresponding to temperature increase (Fig. 2b) in samples subjected to conventional heating with 0% (●), 3% (○), 7% (▼) and 10% (△) fat content. The results are means from three experiments, and error bars indicate standard errors.

fixed temperature and time (60°C, 2 min) is shown in Fig. 7a. The reduction of *Salm*. Typhimurium was not significantly different regardless of fat content. Also, the reduction of *E. coli* O157: H7 and *L. monocytogenes* was not significantly different among fat content levels of 0%, 3% and 7%. However, the reduction of *E. coli* O157: H7 and *L. monocytogenes* decreased when 10% fat content samples were treated with ohmic heating. In contrast with ohmic heating, reductions of all three pathogens subjected to conventional heating did not differ significantly regardless of fat content (Fig. 7b).

Discussion

In conventional heating, heat transfer is achieved via conduction and convection (Zhu *et al.* 2014). Therefore, fat content f samples had no significant effect ($P > 0{\cdot}05$) on heat transfer. The reductions of *E. coli* O157:H7, *Salm*. Typhimurium and *Listeria monocytogenes* were also not significantly different regardless of fat content ($P > 0{\cdot}05$). In addition, the protective effect of fat content on pathogens in the conventionally heated samples at a fixed temperature and time was not observed.

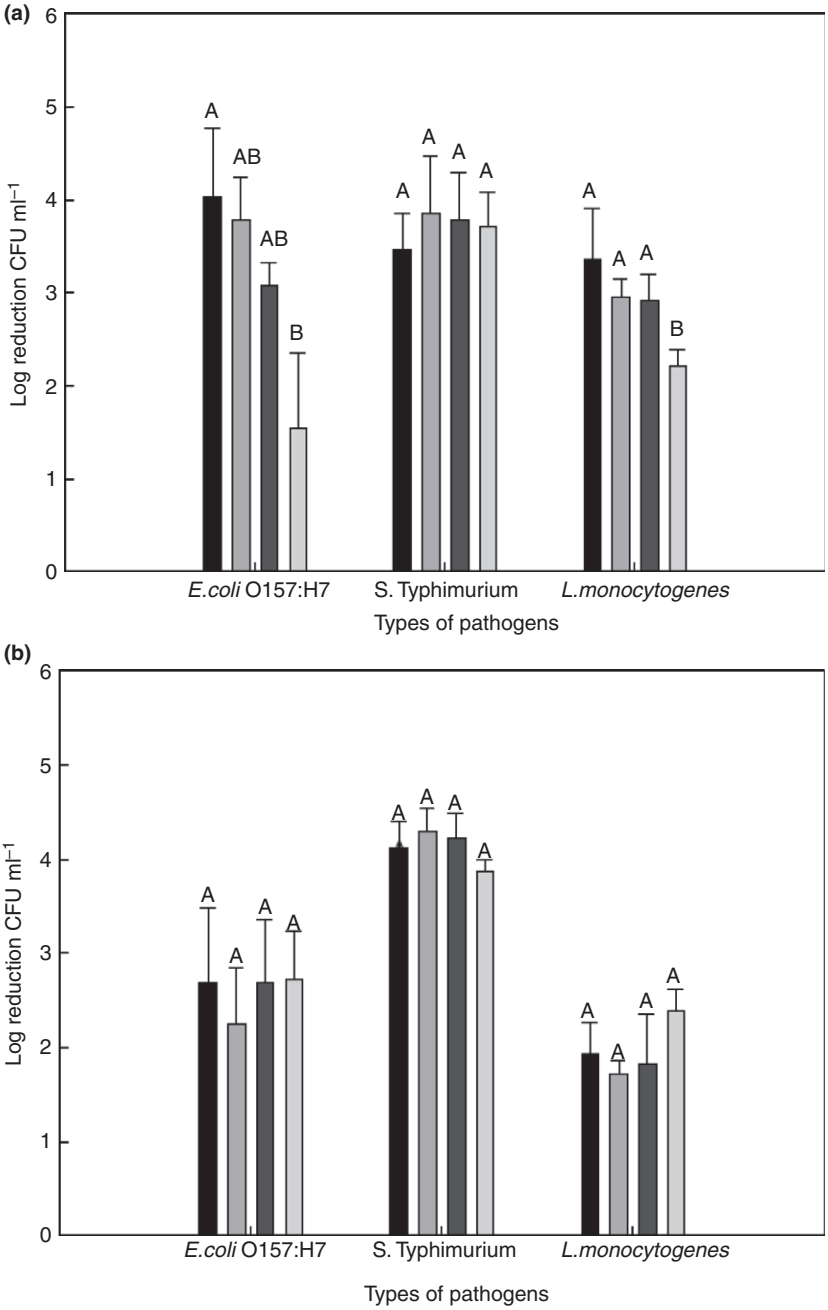

Figure 7 Reduction (log CFU ml^{-1}) of *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* in samples subjected to (a) ohmic and (b) conventional heating at a fixed temperature and time (60°C, 2 min) with 0% (■), 3% (□), 7% (■) and 10% (□) fat content. The results are means from three experiments, and error bars indicate standard errors. Bars for each pathogen with different letters are significantly different ($P < 0{\cdot}05$).

Therefore, it seemed that the inactivation of *E. coli* O157: H7, *Salm*. Typhimurium and *L. monocytogenes* subjected to conventional heating was not significantly affected by the fat content (0–10%). Previous studies also indicated that the reduction of *E. coli* O157:H7 was not significantly influenced ($P > 0{\cdot}05$) by 5–15% and 3–11% fat content when subjected to water bath and oil bath heating, respectively (Kotrola and Conner 1997; Byelashov *et al.* 2010).

In contrast with conventional heating, ohmic heating was significantly affected by fat content (0–10%). In ohmic heating, heat generation is achieved very differently. The success of ohmic heating depends on the rate of heat generation in the system, the electrical conductivity of the food, electrical field strength, residence time and the method by which food flows through the system (Varghese *et al.* 2012). Although many factors can affect the heating rate in an ohmic heating system, generally, the overall electrical resistance of the food system controls the food heating rate. The electrical conductivity of foods is a key parameter of the electrical properties for ohmic heating (Zhu *et al.* 2010).

Lee *et al.* (2013) investigated the effect of frequency on electrical conductivity. Electrical conductivity of the sample increased with increasing frequency. The electrical conductivity of the foodstuff is also highly affected by the food ingredients. Dissolved ingredients such as salts and, acids have an increasing effect on electrical conductivity whereas fat has a decreasing effect (Ramaswamy *et al.* 2014). Bozkurt and Icier (2010) working with minced beef-fat blends, confirmed that samples with lower fat levels had the highest electrical conductivity within tested levels of fat content (2, 9 and 15%). The effect of fat content (0–10%) on electrical conductivity of samples was investigated in the present study. The authors' results indicate that electrical conductivity was higher in the samples with lower fat content. Because of these differences in electrical conductivity, temperature increased more rapidly in the samples having lower fat content. These results suggest that nonuniform heat can be generated by ohmic heating if food contains nonhomogeneous milk fat.

If a fat globule is present within a region of high electrical conductivity, where electric currents can bypass the globule, it may heat more slowly than its surroundings due to its lack of electrical conductivity. Under such conditions, any pathogens potentially present within the fat phase may receive less thermal treatment than the rest of the product (Sastry and Barach 2000). The possibility of nonuniform heat generation was verified using a thermal infrared camera. Marra (2014) suggested that cold areas can exist at junctions of electrodes. Cold areas (blue sections in thermal images of treated samples) were also observed at junctions of electrodes having lateral sample surfaces in the present study. Although only the surface temperature distribution was measured, lack of heating (yellow portion in thermal images) was observed in the 10% fat content samples compared to 0% fat content samples. This difference was more readily identified using enlarged thermal images. Therefore, food-borne pathogens in nonhomogeneous high fat-containing foods subjected to ohmic heating may exhibit increased possibility of survival.

Because the thermal effect is the primary parameter in the inactivation of micro-organisms by ohmic heating (Palaniappan and Sastry 1991; Leizerson and Shimoni 2005), it is a reasonable expectation that pathogens are inactivated more rapidly in samples at higher temperatures. Most of our results agree with this hypothesis, but there were some findings which support the exact opposite. Even though the temperature of 10% fat content samples treated with ohmic heating for 45 s (64·82°C) was slightly higher than that of 7% fat content samples treated for 40 s (63·94°C), the populations of *E. coli* O157: H7 and *L. monocytogenes* were higher in the samples of 7% rather than 10% fat content. Populations of *E. coli* O157: H7 in ohmic heated 10% fat content samples (45 s) and 7% fat content samples (40 s) were 4·06 and 2·93 log CFU ml^{-1}, respectively. Populations of *L. monocytogenes* in ohmic heated 10% fat content samples (45 s) and 7% fat content samples (40 s) were 2·46 and 1·77 log CFU ml^{-1}, respectively. However, this trend was not observed in case of *Salm*. Typhimurium. Populations of *Salm*. Typhimurium in ohmic treated 10% fat content samples (45 s) and 7% fat content samples (40 s) were 1·00 and 1·53 log CFU ml^{-1}, respectively (Fig. 5). These results suggest a protective effect of fat content on *E. coli* O157:H7 and *L. monocytogenes*.

The protective effect was verified by treating samples for a fixed temperature and time. Our results showed that inactivation of *Salm*. Typhimurium was not influenced by fat content (0–10%), whereas survival of *E. coli* O157: H7 and *L. monocytogenes* increased with increasing fat content. Although there were no significant differences among 0, 3 and 7% fat content, the reduction rate decreased when fat content reached 10%. The protective effect of 10% fat content for *E. coli* O157:H7 and *L. monocytogenes* explains why our previous findings (lower reductions of *E. coli* O157:H7 and *L. monocytogenes* in samples subjected to higher temperature) show some interesting and unusual aspects.

The effect of milk fat content on ohmic heating compared to conventional heating was investigated. Milk fat content ranging from 0 to 10% did not have a significant effect on the heating rate of conventionally heated samples. Mostly the protective effect of milk fat on

food-borne pathogens subjected to conventional heating was not observed. On the other hand, milk fat had a significant effect on ohmic heating technology. At first, milk fat content affected the heating rate of ohmic heated samples. Samples with lower fat content had a higher heating rate due to the higher electrical conductivity. As these results indicate the possibility of nonuniform heating, surface temperature distribution was observed using a thermal infrared camera. Also, the protective effect of milk fat on *E. coli* O157:H7 and *L. monocytogenes* was identified. It seemed that milk fat content affected not only the rate of temperature increase but also the survival of some micro-organisms (*E. coli* O157:H7 and *L. monocytogenes*) in the ohmic heated samples. Therefore, milk fat content was identified as an important factor which has a significant effect on the performance of ohmic heating for inactivation of foodborne pathogens.

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Conflict of Interest

None declared.

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